ISSN 2278–540X
EISSN 2278–5396

DRUG DISCOVERY

Ascertaining the protective properties of *Carica Papaya* on the liver of albino wistar rats induced with carbon tetrachloride

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Article History
Received: 03 January 2020
Accepted: 15 February 2020
Published: February 2020

Citation
Kingsley A. Okon, Akpan U. Ekanem, Gabriel D. Edem, Eno-obong B. Ikpeme, Aniebiet A. Udo. Ascertaining the protective properties of *Carica Papaya* on the liver of albino wistar rats induced with carbon tetrachloride. *Drug Discovery*, 2020, 14(33), 44-49

ABSTRACT

Twenty-five (25) mature albino Wistar rats weighing between 175g-200g were divided into five groups of five rats each, based on their body weights. Group A served as the control and received normal feed and clean tap water orally for 7 days. Group B animals received 2.5ml/kg body weight of carbon tetrachloride (CCl_4) on the first day of administration only. Group C animals were administered with 2.5ml/kg body weight of carbon tetrachloride (CCl_4) on the first day of administration and 100mg/kg body weight of the bark (peel) of ripe *carica papaya* extract dissolved in 2ml of distilled water orally, once for 7 days. Group D received 2.5ml/kg body weight of CCL_4 on the first day of administration and 200mg/kg body weight of extract dissolved in 2ml of distilled water orally, once daily for 7 days. Group E received 2.5ml/kg of carbon tetrachloride (CCl_4) on the first day of administration and

300mg/kg body weight of extract dissolved in 2ml of distilled water orally, once daily for 7 days. The animals were sacrificed on the 8th day, the liver removed and processed for Haematoxylin and Eosin staining. Histological results revealed irreversible area of chronic inflammation, sinusoidal enlargement, vascular degeneration, vascular congestion and cellular proliferation with pyknotic nucleus in the treated groups when compared with the control group with normal cellular architecture. This reveals that bark peel of ripe *Carica papaya* fruit exerted little or no hepatoprotective impact on the liver of rats administered with CCl_4.

Keywords: Liver, carbon tetrachloride (CCl_4), *Carica papaya*

1. INTRODUCTION

Carbon tetrachloride is a solvent used in most laboratories for researches, sometimes it gains access to the human body system accidentally or deliberately [1]. Organic solvents are being used frequently in laboratories for drug preparation, preservatives and food production, through these routes; it could get to the human system [1]. Its present in the body may exert helpful and adverse effects on certain key organs of metabolism like the liver, kidney and even on the central nervous system [2]. The liver is mostly affected because it is the key detoxification center where every metabolism is initiated and completed. However, the liver gets dystrophied due to infections (which could be bacterial, viral, protozoan, fungal), auto-immune disease, so the need to protect the liver and guard against liver failure [3].

Carica papaya is a short-lived perennial plant growing to 30ft (9.14m) high. It has fast growing perennial branches up to 10m high with a crown of very large palmate leaves at the base of which the fruits are clustered. The plant is thought to have originated from Mexico and Central America. It has an optional growth condition which is between 21 - 33^{0}C, an annual rainfall of 1200 - 1600mm which requires well drained soils and organic matter rich soils with a pH of about 6.0 - 6.5 [4]. The plant parts rich in phytochemicals include the fruits (both the ripe and unripe), seeds, root and leaves (green, yellow and brown/dry). The above named parts could be used in treating tuberculosis, the sap used to dress wounds, leaves to treat malaria and unripe fruits for typhoid fever [5]. Research has also shown that the *carica papaya* fruit unripe/ripe have wide range of nutrients, vitamins and minerals needed for the normal functioning of the body [6]. The Phytochemical composition of the plant include tannins, alkaloids, flavonoids, cardiac glycosides, phytatis, steroids, as well as papain and chymopapain found in the milky sap, all have their pharmacological effects on the body [6].

2. MATERIALS AND METHOD

Materials Used

Twenty five (25) adult Wistar rats, cages, agro feed (vital grower), stainless plates, sawdust, water bottle, weighing balance, feeding tube, cannula, 250ml beaker, syringes ranging from 1ml to 5ml, glass jar, chopping board, knife, wooden mortar and pestle, white paper, tray, grinder, stirring rod, distilled water, sieve, dissecting set, gloves, molten block, mounting block, slides, coverslips, cotton wool, tissue paper, foil paper, detergents, surgical blade and hot water bath.

Reagents used

Normal saline, 10% buffered formalin, distilled water, chloroform, detol, methylated spirit, 70% alcohol, 95% alcohol, absolute (100%) alcohol, xylene, 1% acid alcohol, Haematoxylin, Eosin, *Carica papaya* fruit and Carbon tetrachlororide (CCl_4).

Handling of Experimental Animals

Twenty five (25) adult Wistar rats were used in this study. They were randomly selected from the animal house of the Faculty of Pharmacy, University of Uyo. The rats were allowed for a period of one week (7 days) to acclimatize to their new environment (Faculty of Basic Medical Sciences Animal House). They were five (5) rats in five labeled wooden cages measuring about 18 by 12inches with wire gauge which covers the cages to serve as a protection and as a source of ventilation for them. Sawdust was used as beddings and vital grower feed which was given to them in stainless plates at least twice a day. The vital grower mash was obtained in bags from Agro Feeds Mills (Nig.) Limited, Uyo outlet. Clean drinking water was also provided ad libitum via water bottles and their cages cleaned twice a week. The rats were exposed to 12 hour light/dark cycle at room temperature. They were identified by different colour markings on their tails based on their respective groupings. The rats were handled and cared for in accordance and in line with the applicable guidelines and standard for the care and use of laboratory animals for animal research study. The rats were weighed with the help of a digital weighing balance before the commencement of every experiment and their weights were determined to fall between 175g-200g.

Extract Preparation

The ripe Paw-paw (*Carica papaya*) fruits were plucked from two different pieces of farm lands located in UruaEkpa Road in Uyo and NungUdoeIbesikpo in IbesikpoAustan Local Government Area, all in AkwaIbom State, Nigeria. The fruits were thoroughly washed, peeled, chopped into small pieces and shade dried. The dried peel was then grounded. To prepare the aqueous extract, 200g of powdered peel of *papaya* fruit was mixed with 1,500ml of distilled water in a conical flask kept for 24hours with occasional shaking and stirring. Filtration was done with the aid of a muslin cloth and the residue separated from the solvent. The solvent was then concentrated with the aid of a hot water bath and stored in a tightly-covered rubber container in a refrigerator.

Experimental Design

The twenty-five rats were randomly selected and used for the study. They were divided into five groups as shown below:

GROUP A: Served as the control and were fed with normal feed and clean tap water orally for 7 days.

GROUP B: Received 2.5ml/kg of carbon tetrachloride (CCl_4) on the first day of administration.

GROUP C: Administered with 2.5ml/kg of carbon tetrachloride (CCl_4) on the first day of administration and 100mg/kg body weight of *carica papaya* peel extract dissolved in 2ml of distilled water orally once daily for 7 days.

GROUP D: Received 2.5ml/kg of carbon tetrachloride (CCl_4) on the first day of administration and 200mg/kg body weight of *carica papaya* peel extract dissolved in 2ml of distilled water orally once daily for 7 days.

GROUP E: Received 2.5ml/kg of carbon tetrachloride (CCl_4) on the first day of administration and 300mg/kg body weight of *carica papaya* peel extract dissolved in 2ml of distilled water orally once daily for 7 days.

Sacrifice

The animals were sacrificed under chloroform anesthesia using chloroform inhalation method. This was done by introducing the animals one after the other into a desiccator in which a cotton wool soaked in chloroform was placed. After inducing the animals into an anesthetic state, they were removed, placed on the dissecting board and dissected. The livers were removed, washed in normal saline to remove excess blood before fixing in 10% buffered formalin, processed and stained using Haematoxylin and Eosin staining method.

3. RESULTS

The following histological features were observed in the various experimental groups of animals.

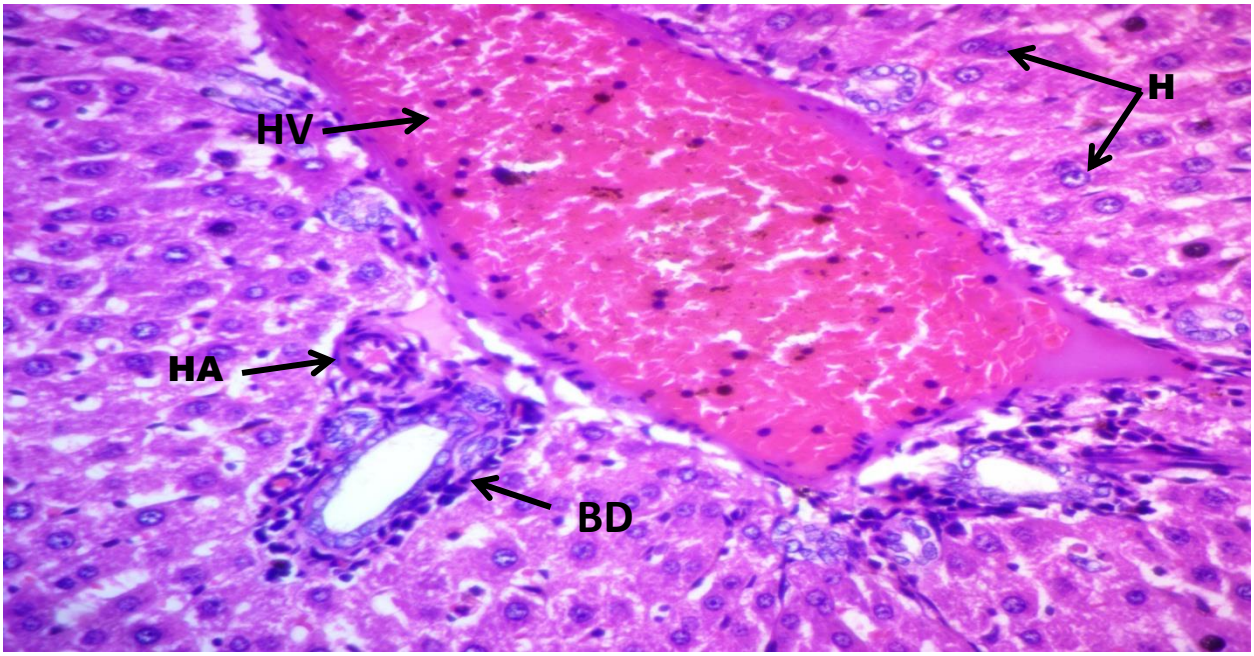

Figure 1 Photomicrograph of the Liver of control rat given distilled water, showing normal histological appearance; Bile duct (**BD**), Hepatic Artery (**HA**), Hepatic vein (**HV**), Hepatocytes (**H**); (H&E x400).

In Group A, this served as the control, the histological photomicrograph revealed normal cellular architecture of portal triad, bile duct, hepatic artery, hepatic vein, hepatocytes and nucleus with normal cellular architecture (Figure 1). In Group B, in which the animals were administered with 2.5ml/kg of carbon tetrachloride (CCl_4) for 1 day, photomicrograph showed chronic inflammation, vascular degeneration, vascular congestion and cellular proliferation with pyknotic nucleus as compared to control group (Figure 2). In Group C, the animals were administered with 2.5ml/kg of carbon tetrachloride (CCl_4) for 1 day and 100mg/kg body weight of *carica papaya* peel extract dissolved in 2ml of distilled water orally once daily for 7 days, photomicrograph revealed irreversible area of Chronic inflammation, vascular degeneration, vascular congestion and cellular proliferation with pyknotic nucleus as compared to control group (Figure 3).

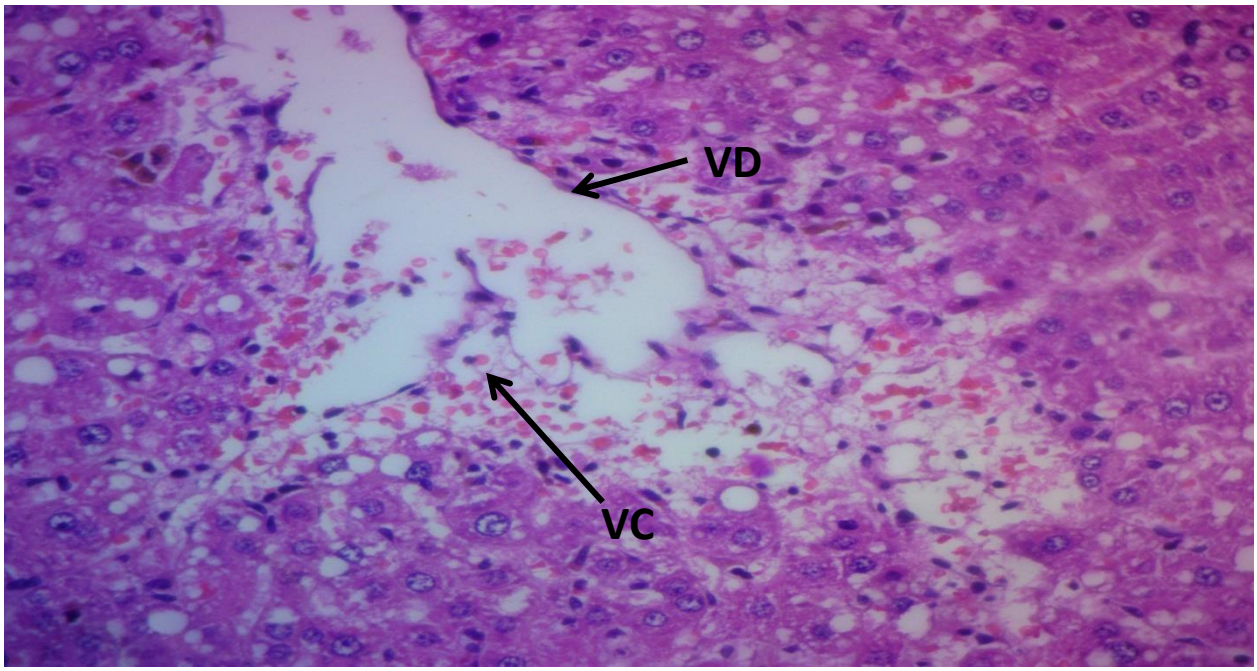

Figure 2 Photomicrograph of the Liver treated with 2.5ml/kg body weight of CCL4 for 1 day showing vascular congestion (**VC**), vascular degeneration (**VD**) (H&E x400).

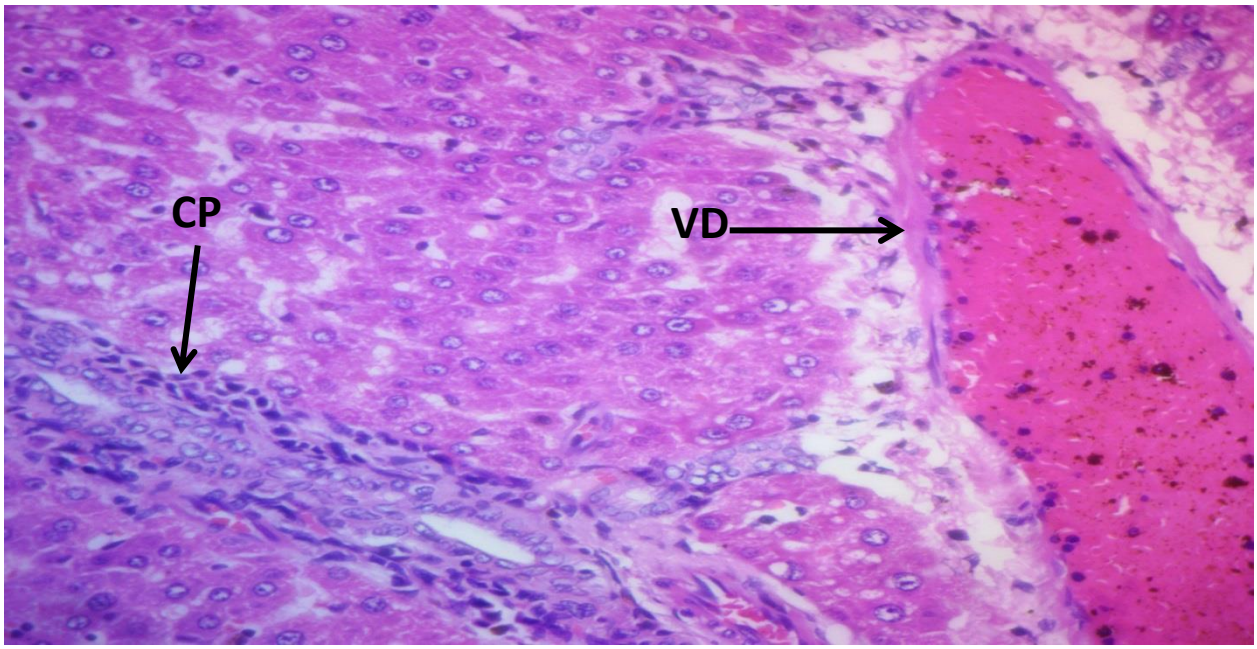

Figure 3 Photomicrograph of the Liver treated with 2.5ml/kg body weight of CCL_4 for 1 day and 100mg/kg of ripe *carica papaya* peel extract for 7 days showingcellular proliferation (**CP**), vascular degeneration (**VD**)(H&E x400).

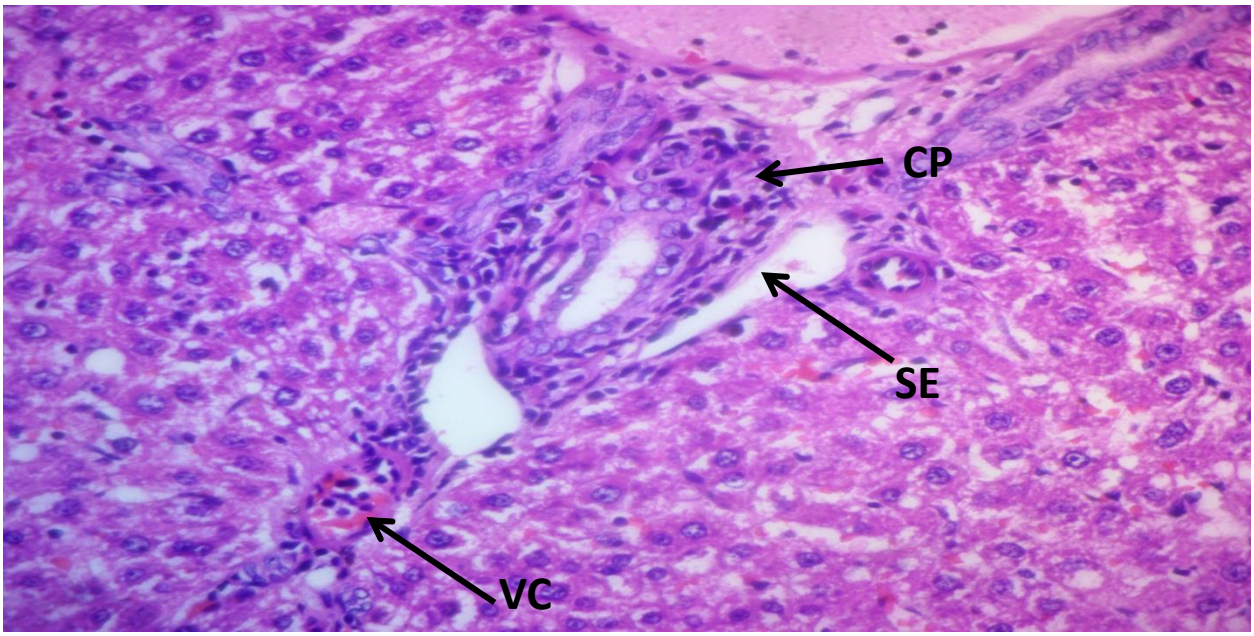

Figure 4 Photomicrograph of the Liver treated with 2.5ml/kg body weight of CCL4 for 1 day and 200mg/kg of ripe *Carica papaya* peel extract for 7 days showing vascular congestion (**VC**), cellular proliferation (CP), and sinusoidal enlargement (SE) (H&E x400).

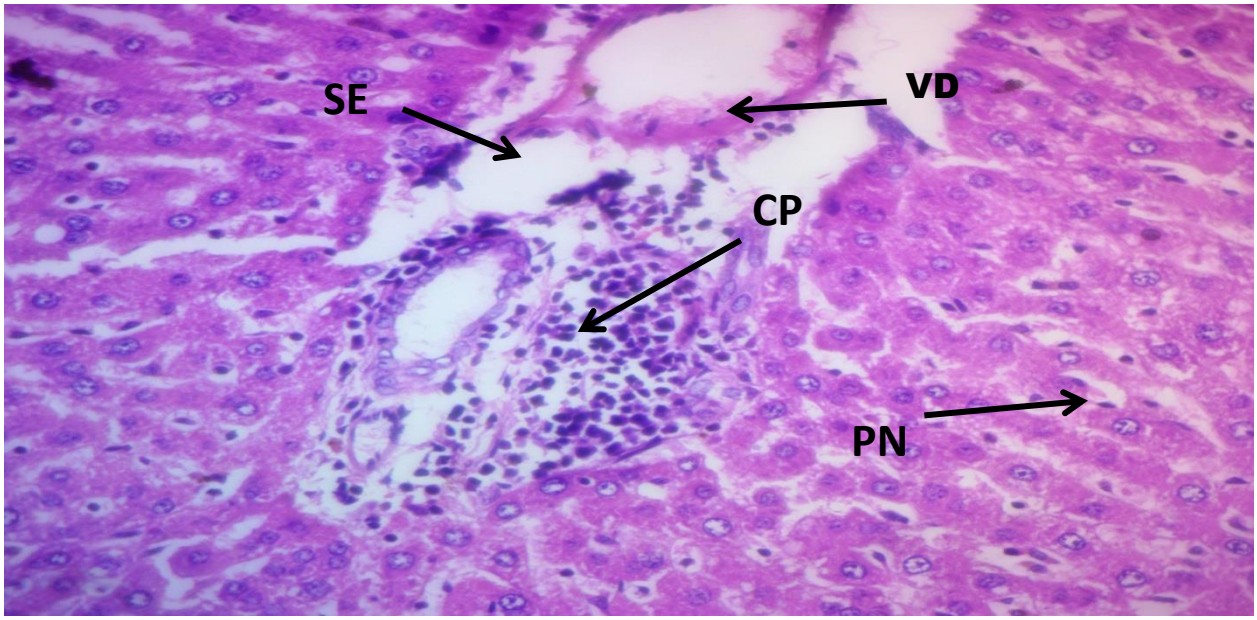

Figure 5 Photomicrograph of the Liver treated with 2.5ml/kg of CCL_4 for 1 day and 300mg/kg of ripe *carica papaya* peel extract for 7 days showing vascular degeneration (**VD)**, cellular proliferation (**CP**), sinusoidal enlargement (**SE**) and pyknotic nucleus (**PN**)(H&E x400).

In Group D, the animals received 2.5ml/kg of Carbon tetrachloride (CCl_4) for 1 day and 200mg/kg body weight of *carica papaya* peel extract dissolved in 2ml of distilled water orally once daily for 7 days, the histologic section of the liver revealed irreversible area of sinusoidal enlargement, vascular congestion and cellular proliferation with pyknotic nucleus as compared to control group (Figure 4). In Group E, the animals were administered with 2.5ml/kg of carbon tetrachloride (CCl_4) for 1 day and 300mg/kg of *carica papaya* peel extract for 7 days, the histologic section of the liver revealed irreversible area of sinusoidal enlargement, vascular degeneration and cellular proliferation with pyknotic nucleus as compared to control group (Figure 5).

4. DISCUSSION

Ripe *Carica papaya* fruit is packed with numerous health benefiting nutrients. The fruit is one of the favorites of fruit-lovers for its nutritional, digestive and medicinal properties; the unripe fruit is used for the treatment of malaria. In this study, it was evident that

administration of 2.5ml/kg body weight of carbon tetrachloride for 1 day is toxic to the liver, with chronic inflammation, vascular degeneration, vascular congestion and cellular proliferation with pyknotic nucleus (Figure 2), compared to the control group. Acute inhalation and oral exposures to high levels of Carbon tetrachloride have been observed primarily to damage the liver (swollen, tender, changes in enzyme levels, jaundice) and kidney (nephritis, nephrosis) of humans [8].

In Groups C, D and E, the histological sections of the liver treated with 2.5ml/kg body weight for 1 day and the administration of 100mg/kg, 200mg/kg and 300mg/kg body weight of ripe *Carica papaya* extract for 7 days respectively revealed irreversible areas of chronic inflammation, vascular degeneration and cellular proliferation with pyknotic nucleus (Figures 3, 4 and 5) as compared to the control group. This is contrary to a report which states that *Carica papaya* fruits apart from being sweet exert some protective effects on the liver against hepatotoxicants like paracetamol (Acetaminophen) [7]. In 2008, Adeneyee and colleques [9] revealed that the protective and ameliorative capacity of the hepatoprotective medicinal plants is actually mediated by the present of flavonoids or alkaloids component or by their combination through antioxidant and free radical scavenging activities. Also, administration of *caruca papaya* fruit aqueous extract of 250mg/kg body weight for forty days significantly lowered lipid peroxidation and enhance glutathione levels, activity of catalase and superoxide dismutase as well as improving immune status reflected in increase IgG and IgM [10]. Another study had also reported that aqueous extracts from both green and ripe *carica papaya* fruit improve wound healing. While the green *carica papaya* extract treatment induced complete healing in shorter period (13 days), the ripe *carica papaya* extract effected healing in 17 days [11]. Therefore, the non-reversible toxic effect cause by CCL_4on the liver in this study despite administration of peel extract of ripe *carica papaya* fruit for 7 days may be due to short period of administration.

5. CONCLUSION

From this investigation, administration of carbon tetrachloride (CCl_4) has been found to be toxic to the liver, while ripe *Carica papaya* fruit is seen to have no reversibility effect and this may be duration dependence.

Funding:
This study has not received any external funding.

Conflict of Interest:
The authors declare that there are no conflicts of interests.

Peer-review:
External peer-review was done through double-blind method.

Data and materials availability:
All data associated with this study are present in the paper.

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